Analysis of Biological Molecules by Single Photon Ionization

Scientific Achievement

Functionalized surfaces serve an important role as interfaces between biological systems and materials. We have developed an analytical method for studying such surfaces by tagging the biological molecule of interest (e.g., peptides) with an ionization-potential (IP) lowering species. The tag allows for efficient and soft photoionization of the molecule with a vacuum ultraviolet laser, so that minute quantities of biological molecules can be measured intact by mass spectrometry.

Significance

Single photon ionization (SPI) has the potential for efficiently and softly ionizing a wide range of molecules for subsequent mass analysis and detection. We have demonstrated this capability on aromatic thiols desorbed from self-assembled monolayer and more recently by softly ionizing amino acids and polypeptides. Soft ionization of these molecules allows us to address a number of important biomaterials systems (for example, functional arrays on biochips or as components of membranes). The detection limits of conventional mass spectrometric methods for measuring peptides are not low enough to be applicable to most biomaterials problems, especially on samples at the nanoscale.

The novel approach employed to ionize these polypeptides (photoionization tagging) requires attaching an electron-withdrawing group before the peptide is attached to the silicon substrate. After the neutral molecule is desorbed from the surface with a laser, the second laser at 157 nm efficiently ionizes the tag species by SPI. In addition to lowering the IP, the tag protects the molecule from extensive fragmentation owing to its ability to localize charge. These phenomena were confirmed by quantum chemical calculations. In many cases, this tag then broke off from the molecule, giving a mass spectrum with the parent ion of interest predominating.

Proteins have also been studied. Insulin is the largest molecule directly photoionized to date (5700 amu), which resulted in an intact molecular ion. It has been widely argued that for molecules larger than about 2000 amu, direct photoionization is impossible. This result demonstrates on the contrary that a wide range of biological molecules may in fact be directly photoionized when a VUV tag is used to permit SPI, leading to highly sensitive detection of the intact molecule with our unique instruments.

This method has been applied to direct measurements of quorum sensing peptides in biofilms. Again it is found that only with the VUV tag and SPI can the peptides be detected with the added benefit that intact molecules are observed.

Performers

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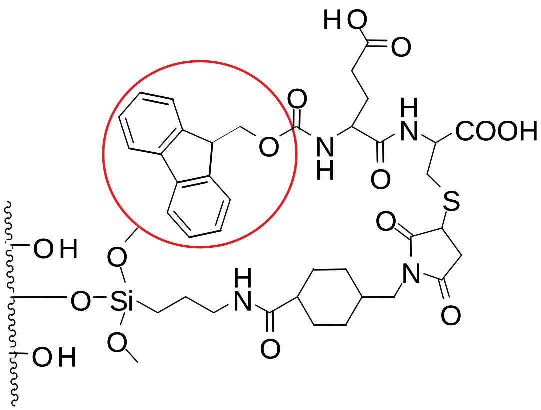
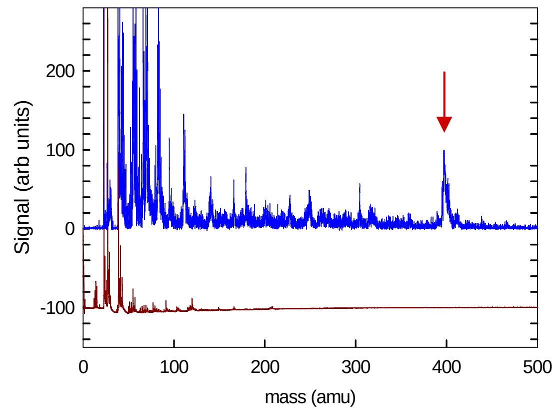
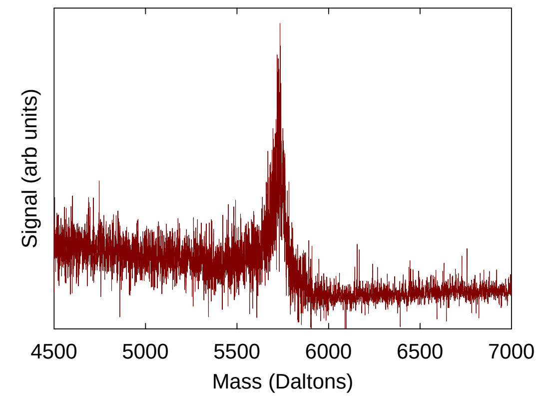
A method was developed to allow very sensitive detection of peptides and proteins by laser desorption followed by vacuum ultraviolet (VUV) single photon ionization.



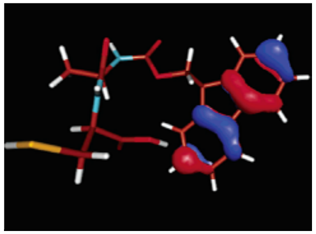
A tag (red circled) attached to the dipeptide lowers the ionization potential to allow single photon ionization.



No molecular ion signal is detected without the tag (bottom). An intact mass peak for the tagged species is observed (red arrow).



Proteins such as insulin (above) were measured using laser desorption coupled with VUV single photon ionization.



Ab-initio calculations (left) show that the cation charge stays primarily on the tag, improving stability of the photoion.

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